

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Publication number:

0 292 846 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **17.08.94** (51) Int. Cl.⁵: **C11C 3/04**, A61K 31/23,
A23D 9/00, A23L 1/30

(21) Application number: **88107910.7**

(22) Date of filing: **18.05.88**

(54) **A process for the extraction of docosahexaenoic acid ethyl ester from fish oils and pharmaceutical and/or dietetic compositions containing a mixture of docosahexaenoic and eicosapentaenoic acid ethyl esters.**

(30) Priority: **28.05.87 IT 2070087**

(43) Date of publication of application:
30.11.88 Bulletin 88/48

(45) Publication of the grant of the patent:
17.08.94 Bulletin 94/33

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(56) References cited:
EP-A- 0 180 786
US-A- 3 082 228
US-A- 4 377 526
US-A- 4 554 107

DERWENT, no. 84-204999, Derwent Publications Ltd, London, GB;& JP-A-59 118 740

PATENT ABSTRACTS OF JAPAN, vol. 300 (C-449)[2747], 29th September 1987;& JP-A-62 93 234

(73) Proprietor: **STAR OIL LTD**
Sceptre House
169/173 Regent Street
London W1R 7FB (GB)

(72) Inventor: **Cornieri, Franco**
Via Lamarmora, 14
I-50121 Firenze (IT)
Inventor: **Di Fulvio, Walter**
Via Lamarmora, 14
I-50121 Firenze (IT)

(74) Representative: **Minoja, Fabrizio**
Studio Consulenza Brevettuale,
Via Rossini, 8
I-20122 Milano (IT)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

Description

The present invention relates to a process for the extraction of docosahexaenoic acid ethyl ester from fish oil, and to pharmaceutical and/or dietetic compositions containing a mixture of eicosapentaenoic and docosahexaenoic acid ethyl esters.

Polyunsaturated fatty acids are known to play two important roles in human physiology: a structural role, as constituents of cell membrane phospholipids, and a functional role, as Prostaglandin precursors.

In fact, fatty acids of the α -linolenic acid family have a basic role in development and function of brain, retina and gonads, as well as the formation of Prostacyclin I_3 (PGI_3) and Thromboxane A_3 (TxA_3), which are factors of paramount importance for the anti-platelet aggregating activity.

Among these, particularly important are the long chain members of the ω -3-family, i.e. eicosapentaenoic ($20:5 \omega - 3$) or EPA and docosahexaenoic ($22:6 \omega - 3$) or DHA acids, deriving from desaturation and elongation of α -linolenic acid, thanks to the intervention of the related enzymes (Δ -desaturase).

EPA, as the precursor of PGI_3 and TxA_3 , exerts an anti-platelet aggregating activity and an anti-thrombotic effect which can be related to cyclooxygenase inhibition (aspirine-like effect) and/or to the competition with arachidonic acid for said enzyme, with an accordingly decreased synthesis of Prostacyclin I_2 (PGI_2) and Thromboxane A_2 (TxA_2), which are known platelet aggregating agents.

DHA is the most important component of human lipids and brain and is present in high concentrations in synaptic membranes phospholipids, which may imply a role in nervous impulse transmission. Moreover, DHA being a structural element of platelet cell, it indirectly exerts an important role in anti-thrombotic action, due to the increase in platelet fluidity.

Recent studies evidenced a decrease in Δ -6 desaturase in man as the age goes on (after 35 years); said phenomenon causes thus an endogenous lack in the above mentioned acids, which therefore should be administered through diet or by means of suitable compositions. However, various practical difficulties opposed up to now a wide use of said acids in therapy or as alimentary integrators, which use on the other hand should be highly desirable, in view of the above reported biochemical and pharmacological considerations.

Said difficulties mainly relate to extraction of said acids from fish oils, purification and concentration to values convenient for the pharmaceutical use and deodorization thereof.

Even though a number of methods have already been proposed and disclosed, the above

objects have still not been attained satisfactorily, as proved, inter alia, by the still restricted use of EPA and/or DHA, in spite of the remarkable potentialities thereof as drugs or alimentary integrators. The methods up to now known, which are based on different techniques such as degreasing, counter-current extraction, urea addition, liquid chromatography, distillation, give rather low yields and products which easily deteriorate if exposed to air or light. Moreover, the major part of the known methods refers to purification of the only eicosapentaenoic acid, to the detriment of other useful unsaturated acids, such as DHA.

As an example, US Patent 4.377.526 discloses a process for purification of EPA or the esters thereof, which comprises treatment with urea, followed by fractional distillation. By said method, EPA percentages higher than 70% are obtained, whereas DHA is present only as a residue (3-5%).

More recently, US Patents 4.554.107 and 4.623.488 disclosed a purification method based on the technique known as molecular distillation: in this case, a deodorized fish oil is obtained which is enriched in EPA and DHA, in rather low yields (30%), due to the drastic conditions used.

A first object of the invention is therefore provided by a method for the extraction of DHA ethyl ester from crude fish oils in high yields, under conditions which can easily be applied on industrial scale, which give a stable and odourless product, which can be used in human therapy or as a dietetic and alimentary integrator.

A second object of the invention, in fact, is provided by pharmaceutical or alimentary compositions containing as the active ingredient DHA ethyl ester, for the treatment or the prophylaxis of cardio-vascular diseases.

According to the present invention, it has been found that highly purified DHA ethyl ester can be obtained starting from crude fish oils, by subjecting them to a transesterification reaction with ethanol, in the presence of sulfuric acid, subsequent silica gel chromatography, treatment of the residue in acetone cooled to -40°C , filtration, evaporation of acetone and two-steps molecular distillation, under controlled conditions.

The process of the invention can be easily carried out on industrial scale, and is characterized in that it consists in a surprisingly low number of steps, if compared with the processes up to now known, which use crude fish oils as the starting material. Moreover, it should be stressed as particularly surprising that enriching in DHA and deodorizing are simultaneously attained by a molecular distillation technique, which has been hitherto considered merely for the purpose of deodorization, with completely different operative parameters.

In fact, the above cited US patents carry out three-steps molecular distillation on long chain unsaturated acid triglycerids, using drastic temperature conditions (up to 260-300 °C), operating in the presence of two additives (glycerol and monooleylglyceride) in order to fluidize the liquid to be distilled.

On the contrary, the process object of the present invention is characterized in that it is carried out on the ethyl ester instead of the triglycerides; moreover operative conditions are much milder (only two steps at a lower temperature) and give higher yields, in DHA which is known to be less stable.

According to the invention, deodorization is effected in the first step and the products responsible for the bad smell are removed by the low temperature trap, upstream the pump. This allows to operate on crude oil not previously depurated, which, besides being effectively deodorized, is also deacidified, to make it suited for alimentary use.

A preferred DHA source consists in oils deriving from working of "blue fish", such as anchovies, sardines, cods, mackerels, herrings and the like.

According to the process of the invention, it is possible to obtain docosahexaenoic acid having an assay as high as 85-95%, according to the starting fish oil used. For this purpose, the oil is diluted with ethanol, then refluxed in the presence of catalytic amounts of concentrated sulfuric acid. After extraction with hexane, the transesterification mixture is subjected to silica gel chromatography, then the ester mixture from the silica gel chromatography is dissolved in acetone and the solution slowly cooled to -40 °C. The formed precipitate is then filtered, the solvent is removed under reduced pressure and the residue is subjected to molecular distillation which is carried out in two steps, with a vacuum of 0.133 Pa (10^{-3} torr), and at an evaporation temperature of 80/100 °C for the first step and 105/125 °C for the second step.

The product obtained by the process of the invention proved to be particularly convenient for pharmaceutical use, in form of appropriate pharmaceutical compositions. In fact, a favourable synergism was evidenced between EPA and DHA, such as to give a therapeutical effectiveness higher than that of the single components. The pharmaceutical compositions of the invention will be prepared by means of techniques and excipients conventionally used for active ingredients in form of oils, as described in "Remington's Pharmaceutical Sciences handbook", Hack Pub. Co., N.Y. USA. Preferred administration routes are the oral and the parenteral ones, whereas posology will generally range from 500 to 5.000 mg of EPA and DHA ethyl ester mixture obtained by the inventive process, depending on pathology and conditions of the pa-

tient to be treated. Anyway, higher dosages are not contraindicated, since the active ingredient is almost non-toxic. The same mixture can be used as dietetic or alimentary integrator, optionally diluted with other appropriate vegetal oils.

In fact, the mixture is particularly convenient for the prophylaxis of diseases related to platelet hyperaggregation conditions, since it is completely free from linolenic acid derivatives which are precursors of arachidonic acid and accordingly of Prostaglandin E_2 (PGE_2) and TxA_2 which are factors able to oppose and make void the favourable pharmacologic properties deriving from the production of PGI_3 and TxA_3 , induced by EPA, DHA and derivatives thereof.

Claims

**Claims for the following Contracting States :
AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE**

1. A process for the extraction of docosahexaenoic acid ethyl ester from fish oil, comprising transesterification of the fish oil with ethanol, in the presence of sulfuric acid, subsequent extraction of the mixture with hexane, silica gel chromatography, treatment of the residue in acetone cooled to -40 °C, filtration, evaporation of acetone and two-steps molecular distillation at 0.133 Pa (10^{-3} torr), the first step at a temperature of 80-100 °C and the second step at a temperature of 105-125 °C.
2. Pharmaceutical and/or alimentary compositions containing a mixture of eicosapentaenoic and docosahexaenoic acid ethyl esters.

**Claims for the following Contracting States :
ES, GR**

1. A process for the extraction of docosahexaenoic acid ethyl ester from fish oil, comprising transesterification of the fish oil with ethanol, in the presence of sulfuric acid, subsequent extraction of the mixture with hexane, silica gel chromatography, treatment of the residue in acetone cooled to -40 °C, filtration, evaporation of acetone and two-steps molecular distillation at 0.133 Pa (10^{-3} torr), the first step at a temperature of 80-100 °C and the second step at a temperature of 105-125 °C.

Patentsprüche

**Patentsprüche für folgende Vertragsstaaten :
AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE**

1. Verfahren zur Extraktion Von Docosahexaensäure-ethylester aus Fischöl, umfassend die Umesterung des Fischöls mit Ethanol in Anwe-

senheit von Schwefelsäure, die anschließende Extraktion des Gemischs mit Hexan, Kieselgel-Chromatographie, Behandeln des Rückstands in auf -40°C gekühltem Aceton, Filtration, Verdampfen des Acetons und zweistufige Molekulardestillation bei 0,133 Pa (10^{-3} torr), die erste Stufe bei einer Temperatur von $80 - 100^{\circ}\text{C}$ und die zweite Stufe bei einer Temperatur von $105 - 125^{\circ}\text{C}$.

2. Pharmazeutische und/oder Nahrungsmittel-Zusammensetzungen, enthaltend ein Gemisch aus Eicosapentaen- und Docosahexaen-säure-ethylestern.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Extraktion von Docosahexaensäure-ethylester aus Fischöl, umfassend die Umesterung des Fischöls mit Ethanol in Anwesenheit von Schwefelsäure, die anschließende Extraktion des Gemischs mit Hexan, Kieselgel-Chromatographie, Behandeln des Rückstands in auf -40°C gekühltem Aceton, Filtration, Verdampfen des Acetons und zweistufige Molekulardestillation bei 0,133 Pa (10^{-3} torr), die erste Stufe bei einer Temperatur von $80 - 100^{\circ}\text{C}$ und die zweite Stufe bei einer Temperatur von $105 - 125^{\circ}\text{C}$.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Procédé pour l'extraction de l'ester éthylique de l'acide docosahexénoïque à partir d'huile de poisson, comprenant une transestérification de l'huile de poisson avec de l'éthanol, en présence d'acide sulfurique, une extraction subséquente du mélange avec de l'hexane, une chromatographie sur gel de silice, un traitement du résidu dans de l'acétone refroidie à -40°C , une filtration, l'évaporation de l'acétone et une distillation moléculaire en deux étapes à 0,133 Pa (10^{-3} torr), la première étape à une température de $80 - 100^{\circ}\text{C}$ et la deuxième étape à une température de $105 - 125^{\circ}\text{C}$.
2. Compositions pharmaceutiques et/ou alimentaires contenant un mélange des esters éthyliques des acides eicosapenténoïque et docosahexénoïque.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé pour l'extraction de l'ester éthylique de l'acide docosahexénoïque à partir d'huile de poisson, comprenant une transestérification de l'huile de poisson avec de l'éthanol, en présence d'acide sulfurique, une extraction subséquente du mélange avec de l'hexane, une chromatographie sur gel de silice, un traitement du résidu dans de l'acétone refroidie à -40°C , une filtration, l'évaporation de l'acétone et une distillation moléculaire en deux étapes à 0,133 Pa (10^{-3} torr), la première étape à une température de $80 - 100^{\circ}\text{C}$ et la deuxième étape à une température de $105 - 125^{\circ}\text{C}$.